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Fulbright & Jaworski L.L.P.

Laurie Oles

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Thierry Boon-Falleur, et al.
Serial No.	:	09/782,745
Filed	:	February 13, 2001
For	:	ISOLATED NUCLEIC ACID MOLECULES WHICH ENCODE GAGE GENES AND USES THEREOF
Group Art Unit	:	1644
Examiner	:	M. DiBrino

October 30, 2002

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

SHOWING OF CHANGES
(37 CFR §1.121(h))

Page 2, lines 1-6:

This application is a divisional of Serial No. 09/012,818, filed January 28, 1998, now abandoned, which is a continuation-in-part of Serial No. 08/531,662, filed September 21, 1995, which is a continuation-in-part of copending application Serial No. 08/370,648, filed January 10, 1995, which is a continuation-in-part of copending Serial No. 08/250,162 filed on May 27, 1994, which is a continuation-in-part of Serial No. 08/096,039, filed July 22, 1993. All of these applications are incorporated by reference.

Page 4, lines 9-23:

In U.S. Patent Application Serial Number 008,446, filed January 22, 1993, now U.S. Patent No. 5,558,995 and incorporated by reference, the fact that the MAGE-1 expression product is processed to a second TR is disclosed. This second TRA is presented by HLA-C clone 10 molecules. The disclosure shows that a given TRAP can yield a plurality of TRAs.

U.S. Patent Application Serial Number 994,928, filed December 22, 1992, now abandoned, and incorporated by reference herein teaches that tyrosinase, a molecule which is produced by some normal cells (e.g., melanocytes), is processed in tumor cells to yield peptides presented by HLA-A2 molecules.

In U.S. Patent Application Serial Number 08/032,978, now U.S. Patent No. 5,620,886, filed March 18, 1993, and incorporated by reference in its entirety, a second TRA, not derived from tyrosinase is taught to be presented by HLA-A2 molecules. The TRA is derived from a TRAP, but is coded for by a non-MAGE gene. This disclosure shows that a particular HLA molecule may present TRAs derived from different sources.

In U.S. Patent Application Serial Number 08/079,110, now U.S. Patent 5,571,711, filed June 17, 1993, and incorporated by reference herein, an unrelated tumor rejection antigen precursor, the so-called "BAGE" precursor, is described. The BAGE precursor is not related to the MAGE family.

Page 5, lines 8-14:

Thus, another feature of the invention are peptides which are anywhere from 9 to 16 amino acids long, and comprise the sequence:

Xaa Xaa Trp Pro Xaa Xaa Xaa Xaa Tyr

(SEQ ID NO: 23)

where Xaa is any amino acid [and $Xaa_{(1,2)}$ means that 1 or 2 amino acids may be N-terminal to the Trp residue]. These peptides bind to, and/or are processed to peptides which bind to HLA-A29 molecules.

The invention is elaborated upon further in the disclosure which follows.

Page 5, lines 21.22:

Figure 3 compares lysis induced by cytolytic T lymphocytes of clone CTL 76/6. Peptides of varying length were tested, including SEQ ID NOS: 4, 5, 6, 7, 8 and 12.

Page 6, lines 1-17:

Figure 4 presents an alignment of the cDNAs of the six GAGE genes discussed herein. In the figure, identical regions are surrounded by boxes. Translation initiation sites and stop codons are also indicated. Primers, used in polymerase chain reaction as described in the examples, are indicated by arrows. SEQ ID NOS: 1, 14, 15, 16, 17 and 18 are set out.

Figure 5 sets forth the alignment of deduced amino acid sequences for the member of the GAGE family. Identical regions are shown by boxes, and the antigenic peptide of SEQ ID NO: 4 is shown. SEQ ID NOS: 26, 27, 28, 29, 30 and 31 are set out.

Figure 6 shows the results obtained when each of the GAGE cDNAs was transfected into COS cells, together with HLA-Cw6 cDNA. Twenty-four hours later, samples of CTL 76/6 were added, and TNF release was measured after twenty-four hours.

Figure 7 compares the stimulation of CTL 22/23 by COS-7 cells, transfected with HLA-A29 cDNA, a MAGE, BAGE or GAGE sequence, as shown. Control values are provided by MZ2-MEL.43 and COS cells, as stimulators.

Figure 8 presents results obtained by ^{51}Cr release studies, using various peptides including SEQ ID NO: 22 and various peptides derived therefrom. The peptides are amino acids 5-12, 4-12, 3-12, 6-13, 5-13, and 4-13 of SEQ ID NO: 21.

Page 7, lines 9-18:

The lysis assay employed was a chromium release assay following Herin et at., Int. J. Cancer 39:390-396 (1987), the disclosure of which is incorporated by reference. The assay, however, is described herein. The target melanoma cells were grown in vitro, and then resuspended at 10^7 cells/ml in DMEM, supplemented with 10mM [HEPES] Hepes and 30% FCS, and incubated for 45 minutes at 37°C with 200 μCi/ml of $Na(^{51}Cr)O_4$. Labeled cells were washed three times with DMEM supplemented with 10 mM Hepes. These were then resuspended in DMEM supplemented with 10mM Hepes and 10% FCS, after which 100 ul aliquots containing 10^3 cells, were distributed into 96 well microplates. Samples of PBLs were added in 100 ul of the same medium, and assays were carried out in duplicate. Plates were centrifuged for 4 minutes at 100g, and incubated for four hours at 37°C in an 8% CO_2 atmosphere.

Page 9, lines 22-page 10, line 10

Following preparation of the library described in Example 3, the cDNA was transfected into eukaryotic cells. The transfections, described herein, were carried out in duplicate. Samples of COS-7 cells were seeded, at 15,000 cells/well into tissue culture flat bottom microwells, in Dulbecco's modified Eagles Medium ("DMEM") supplemented with 10% fetal calf serum. The cells were incubated overnight at 37°C, medium was removed and then replaced by 50 μl/well of DMEM medium containing 10% Nu serum, 400 μg/ml DEAE-dextran, and 100 μM

chloroquine, plus 100 ng of the plasmids. As was indicated supra, the lysis studies did not establish which HLA molecule presented the antigen. As a result, cDNA for each of the HLA molecules which could present the antigen (A1, B37, Cw6) was used, separately, to cotransfect the cells. Specifically, one of 28 ng of the gene encoding HLA-A1 cloned into pCD-SRα, 50 ng of cDNA for HLA-B37 in pcDNA I/Amp, or 75 ng of cDNA for HLA-Cw6 in pcDNAI-Amp, using the same [protocol] protocols as were used for transfection with the library.

Page 12, lines 1-4:

Following sequencing of the cDNA, as per Example 6, experiments were carried out to determine if cells of normal tissues expressed the gene. To determine this, Northern blotting was carried out on tissues and tumor cell lines, as indicated below. The blotting experiments used cDNA for the complete sequence of SEQ ID NO: 1. [PCT] PCR was then used to confirm the results.

Page 16, lines 5-14:

The complete cDNA of GAGE in expression vector pcDNA/AmpI was digested with restriction endonucleases NotI and SpHI, and then with exonucleuase III following supplier's instruction (Erase-a-base System, Promega). This treatment generated a series of progressive deletions, staring at the 3' end.

The deletion products were ligated back into pcDNA/AmpI, and then electroporated into E.coli strain DH5α'IQ, using well known techniques. The transformants were selected with ampicillin (50 micrograms/ml).

Plasmid DNA was extracted from each recombinant clone and was then transfected into COS-7 cells, together with a vector which coded for HLA-Cw6. The protocols used [follow] followed the protocols described above.

Page 20, lines 8-13:

The deduced GAGE-1 protein corresponding to a tumor rejection antigen precursor is about 20 amino acids longer than the 5 other proteins, whose last seven residues also differ from the homologous residues of GAGE-1 (Figure 5). The rest of the protein sequences show only 10 mismatches. One of these is in the region corresponding to the antigenic peptide of SEQ ID NO: 4. The sequence of the peptide is modified in GAGE-3, 4, 5 and 6 so that position 2 is now W instead of R.

Page 26, lines 1-3:

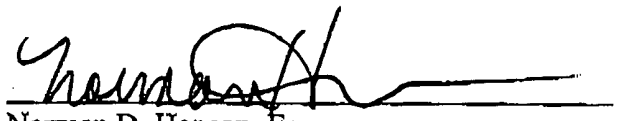
This databas is available at the NCBI (USA), or on Web [Solte] Site (Internet) WWW.NCBI.NLM.NIH.GOV. The filters were washed twice at room temperature for 5 minutes each time, at 6xSSC, followed by two washes, at 6xSSC (5 minutes per wash), at 42°C.

Page 32, lines 2-11:

Especially preferred are peptides which, in accordance with the formula of SEQ ID NOS: 23, 24 and 25 also satisfy one or more of the following cirteria: the N-terminal amino acid position is Tyr, second position is Tyr, fourth position is Pro, fifth position is Arg, sixth position is Pro, seventh position is Arg, and eighth position is Arg. Of course, the fourth position is already fixed in SEQ ID NO; 23, the fifth is already fixed in SEQ ID NO: 24, and the eighth in SEQ ID NO: 25. When all of these criteria are satisfied and this peptide consists of 9 amino acids, one has SEQ ID NO: 22. Any or al of the foreign specific alternatives may be combined in the peptides of the claimed invention, subject to the motif of SEQ ID NO: 23, 24 or 25 and the size of 9-16 amino acids. Especially preferred are peptides which are 9-14 amino acids long, and which include SEQ ID NO: 23, 24 or 25, subject to the above preferred alternatives.

Respectfully submitted,

FULBRIGHT & JAWORSKI, L.L.P.



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Fulbright & Jaworski L.L.P.

Dawn Oleb

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Thierry Boon-Falleur, et al.
Serial No.	:	09/782,745
Filed	:	February 13, 2001
For	:	ISOLATED NUCLEIC ACID MOLECULES WHICH ENCODE GAGE GENES AND USES THEREOF
Group Art Unit	:	1644
Examiner	:	M. DiBrino

October 30, 2002

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

AMENDMENT
UNDER 37 CFR §1.111

This amendment is submitted in response to the office action dated August 9, 2002. Please amend this application as follows:

IN THE FIGURES

Please replace figures 3, 4, 5 & 8 with the attached.

IN THE SPECIFICATION

Page 2, lines 1-6: replace by:

This application is a divisional of Serial No. 09/012,818, filed January 28, 1998, now abandoned, which is a continuation-in-part of Serial No. 08/531,662, filed September 21, 1995, which is a continuation-in-part of copending application Serial No. 08/370,648, filed January 10, 1995, which is a continuation-in-part of copending Serial No. 08/250,162 filed on May 27, 1994,

C1

C1 which is a continuation-in-part of Serial No. 08/096,039, filed July 22, 1993. All of these applications are incorporated by reference.

Page 4, lines 9-line 23: replace by:

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Page 5, lines 8-14: replace by:

Thus, another feature of the invention are peptides which are anywhere from 9 to 16 amino acids long, and comprise the sequence:

Xaa Xaa Trp Pro Xaa Xaa Xaa Xaa Tyr

C3 (SEQ ID NO: 23)

where Xaa is any amino acid. These peptides bind to, and/or are processed to peptides which bind to HLA-A29 molecules.

Page 5, lines 21-22: replace by:

Figure 3 compares lysis induced by cytolytic T lymphocytes of clone CTL 76/6. Peptides of varying length were tested, including SEQ ID NOS: 4, 5, 6, 7, 8 and 12.

Page 6, lines 1-17: replace by:

Figure 4 presents an alignment of the cDNAs of the six GAGE genes discussed herein. In the figure, identical regions are surrounded by boxes. Translation initiation sites and stop codons are also indicated. Primers, used in polymerase chain reaction as described in the examples, are indicated by arrows. SEQ ID NOS: 1, 14, 15, 16, 17 and 18 are set out.

Figure 5 sets forth the alignment of deduced amino acid sequences for the member of the GAGE family. Identical regions are shown by boxes, and the antigenic peptide of SEQ ID NO: 4 is shown. SEQ ID NOS: 26, 27, 28, 29, 30 and 31 are set out.

C4

Figure 6 shows the results obtained when each of the GAGE cDNAs was transfected into COS cells, together with HLA-Cw6 cDNA. Twenty-four hours later, samples of CTL 76/6 were added, and TNF release was measured after twenty-four hours.

Figure 7 compares the stimulation of CTL 22/23 by COS-7 cells, transfected with HLA-A29 cDNA, a MAGE, BAGE or GAGE sequence, as shown. Control values are provided by MZ2-MEL.43 and COS cells, as stimulators.

Figure 8 presents results obtained by ^{51}Cr release studies, using various peptides including SEQ ID NO: 22 and various peptides derived therefrom. The peptides are amino acids 5-12, 4-12, 3-12, 6-13, 5-13, and 4-13 of SEQ ID NO: 21.

Page 7, lines 9-18: replace by:

The lysis assay employed was a chromium release assay following Herin et at., Int. J. Cancer 39:390-396 (1987), the disclosure of which is incorporated by reference. The assay , however, is described herein. The target melanoma cells were grown in vitro, and then resuspended at 10^7 cells/ml in DMEM, supplemented with 10mM Hepes and 30% FCS, and incubated for 45 minutes at 37°C with 200 μCi/ml of $Na(^{51}Cr)O_4$. Labeled cells were washed three times with DMEM supplemented with 10 mM Hepes. These were then resuspended in DMEM supplemented with 10mM Hepes and 10% FCS, after which 100 ul aliquots containing 10^3 cells, were distributed into 96 well microplates. Samples of PBLs were added in 100 ul of the same medium, and assays were carried out in duplicate. Plates were centrifuged for 4 minutes at 100g, and incubated for four hours at 37°C in an 8% CO_2 atmosphere.

C5

Page 9, lines 22-page 10, line 10: replace by:

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C6

Dulbecco's modified Eagles Medium ("DMEM") supplemented with 10% fetal calf serum. The cells were incubated overnight at 37°C, medium was removed and then replaced by 50 µl/well of DMEM medium containing 10% Nu serum, 400 µg/ml DEAE-dextran, and 100 µM chloroquine, plus 100 ng of the plasmids. As was indicated supra, the lysis studies did not establish which HLA molecule presented the antigen. As a result, cDNA for each of the HLA molecules which could present the antigen (A1, B37, Cw6) was used, separately, to cotransfect the cells. Specifically, one of 28 ng of the gene encoding HLA-A1 cloned into pCD-SRα, 50 ng of cDNA for HLA-B37 in pcDNA I/Amp, or 75 ng of cDNA for HLA-Cw6 in pcDNAI-Amp, using the same protocols as were used for transfection with the library.

C6

Page 12, lines 1-4: replace by:

Following sequencing of the cDNA, as per Example 6, experiments were carried out to determine if cells of normal tissues expressed the gene. To determine this, Northern blotting was carried out on tissues and tumor cell lines, as indicated below. The blotting experiments used cDNA for the complete sequence of SEQ ID NO: 1. PCR was then used to confirm the results.

C7

Page 16, lines 5-14: replace by:

The complete cDNA of GAGE in expression vector pcDNA/AmpI was digested with restriction endonucleases NotI and SpHI, and then with exonucleuase III following supplier's instruction (Erase-a-base System, Promega). This treatment generated a series of progressive deletions, staring at the 3' end.

C8

The deletion products were ligated back into pcDNA/AmpI, and then electroporated into E.coli strain DH5α'IQ, using well known techniques. The transformants were selected with ampicillin (50 micrograms/ml).

Plasmid DNA was extracted from each recombinant clone and was then transfected into COS-7 cells, together with a vector which coded for HLA-Cw6. The protocols used followed the protocols described above.

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C9

C9 4. The sequence of the peptide is modified in GAGE-3, 4, 5 and 6 so that position 2 is now W instead of R.

Page 26, lines 1-3: replace by:

This database is available at the NCBI (USA), or on Web Site (Internet)

C10 WWW.NCBI.NLM.NIH.GOV. The filters were washed twice at room temperature for 5 minutes each time, at 6xSSC, followed by two washes, at 6xSSC (5 minutes per wash), at 42°C.

Page 32, lines 2-11: replace by:

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C11 23, 24 and 25 also satisfy one or more of the following cirteria: the N-terminal amino acid position is Tyr, second position is Tyr, fourth position is Pro, fifth position is Arg, sixth position is Pro, seventh position is Arg, and eighth position is Arg. Of course, the fourth position is already fixed in SEQ ID NO; 23, the fifth is already fixed in SEQ ID NO: 24, and the eighth in SEQ ID NO: 25. When all of these criteria are satisfied and this peptide consists of 9 amino acids, one has SEQ ID NO: 22. Any or al of the foreign specific alternatives may be combined in the peptides of the claimed invention, subject to the motif of SEQ ID NO: 23, 24 or 25 and the size of 9-16 amino acids. Especially preferred are peptides which are 9-14 amino acids long, and which include SEQ ID NO: 23, 24 or 25, subject to the above preferred alternatives.

IN THE CLAIMS

Cancel claims 32-40 without prejudice.

Add claims 41-50, which follow:

Claim 41: An isolated nucleic acid molecule which encodes a GAGE tumor rejection antigen precursor, the nucleotide sequence of which is set forth in SEQ ID NO: 14, 15, 16, 17, or 18.

Claim 42: An expression vector useful in producing a GAGE tumor rejection antigen, comprising the isolated nucleic acid molecule of claim 41, operably linked to a promoter.

C12

Claim 43: An isolated cell, transformed or transfected with the isolated nucleic acid molecule of claim 41.

Claim 44: An isolated cell, transformed or transfected with the expression vector of claim 42.